

B13 The saccharin-terminated (SEQ ID NO: 4) BHA.Lys.Lys<sub>2</sub>Lys<sub>4</sub>.Lys<sub>8</sub>.Lys<sub>16</sub>.Lys<sub>32</sub>... dendrimer  
BRI-6189 was similarly prepared.

N/A After page 59, insert the printed Sequence Listing.

**REMARKS**

Applicants submit this Amendment to indicate the insertion point for the substitute Sequence Listing filed concurrently herewith. Applicants respectfully request examination on the merits of this application.

Receipt of the initial Office Action on the merits is awaited.

Respectfully submitted,

July 12, 2001  
Date

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**Versions with Markings to Show Changes Made**

**IN THE SPECIFICATION**

Please amend the Specification as follows:

Please replace the paragraph beginning on page 13 at line 25 with the following rewritten paragraph:

Further features of the present invention will be apparent from the following Examples which are included by way of illustration, not limitation of the invention. In the following Examples, PAMAM dendrimers refer to polyamidoamine dendrimers based on an ammonia core as detailed in U.S. Patents Nos. 4,507,466, 4,558,120, 4,568,737 and 4,587,329; PAMAM (EDA) dendrimers refer to polyamidoamine dendrimers based on an ethylene diamine core; and BHAlys<sub>x</sub>lys<sub>y</sub>lys<sub>z</sub> dendrimers refer to polylysine unsymmetrical dendrimers based on a benzhydrylamine core and lysine branching units as described in US Patents Nos. 4,289,872 and 4,410,688. The polyamidoamine dendrimers PAMAM 1.0, PAMAM 2.0, PAMAM 3.0, PAMAM 4.0, PAMAM 5.0 or higher generation, PAMAM 4.0 (EDA), and the polylysine dendrimers BHAlyslys<sub>2</sub>, (SEQ ID NO: 1) BHAlyslys<sub>2</sub>lys<sub>4</sub>, (SEQ ID NO. 2) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub> and (SEQ ID NO. 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>, (SEQ ID NO. 4) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub>, (SEQ ID No. 5) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub>lys<sub>64</sub>, or higher generations prepared as described in US Patents Nos. 4289872, 4410688, 4507466, 4558120, 4568737 and 4578239 and International Patent Publications Nos. WO 88/01178, WO 88/01179, WO 88/01180 and WO 95/24221 referred to above.

Please replace the paragraph beginning on page 21 at line 14 with the following rewritten paragraph:

Preparation of sodium N-(2-sulfoethyl) succinamide terminated polylysine dendrimers (SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>, **BRI2789**

Trifluoroacetic acid (1ml) was added to a suspension of (SEQ ID NO: 2) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>DBL<sub>6</sub> (36.5mg; 5.0μmol) in dry dichloromethane (1ml) and the resulting solution stirred at room temperature under nitrogen for two hours and then concentrated. The residue was dissolved in dry DMSO (2ml) and the pH adjusted to 8.5 with triethylamine. A

solution of the crude tetrabutylammonium 4-nitrophenyl N-(2-sulfoethyl)succinamate (ca. 0.2mmol) in DMSO (1ml) was then added dropwise and the mixture stirred overnight at room temperature. The yellow solution was then concentrated (50 /10<sup>-5</sup> mmHg) and the yellow residue partitioned between water and chloroform. The aqueous layer was separated, washed with chloroform (3X) and ethyl acetate, and then concentrated to give an oil (99mg). The crude product was converted to the sodium salt by passage through a column of Amberlite IR 120(Na) to yield 81 mg of material. This material was further purified by gel filtration (Sephadex LH20; water) to give the sodium N-(2-sulfoethyl)succinamide terminated (SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> dendrimer (39mg). <sup>13</sup>C nmr(D<sub>2</sub>O):δ 27.0, 32.3, 35.2, 35.3, 35.6, 35.7, 39.5, 43.5, 54.1, 58.5, 131.5, 132.0, 133.3, 145.1, 177.8, 178.0, 178.4, 178.8, 178.9, 179.2, 179.7, 179.8.

Please replace the paragraphs beginning on page 22 at lines 4, with the following rewritten paragraph:

The corresponding (SEQ ID NO: 1) BHAlyslys<sub>2</sub>, BHAlyslys<sub>2</sub>lys<sub>4</sub> (BRI2787) and (SEQ ID NO: 2) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub> (BRI2788) terminated with sodium N-(2-sulfoethyl)succinamide groups were similarly prepared. <sup>13</sup>C nmr (SEQ ID NO: 2) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub> derivative (D<sub>2</sub>O):δ 26.9, 32.3, 35.1, 35.3, 35.6, 35.7, 39.5, 43.5, 54.1, 58.5, 131.6, 131.9, 132.2, 132.3, 133.2, 133.3, 145.0, 145.2, 177.2, 177.8, 177.9, 178.0, 178.2, 178.3, 178.6, 178.7, 178.8, 178.9, 179.2, 179.3, 179.7, 179.8.

<sup>13</sup>C nmr (SEQ ID NO: 1) BHAlyslys<sub>2</sub>lys<sub>4</sub> derivative (D<sub>2</sub>O):δ 26.9, 32.3, 35.1, 35.4, 35.7, 35.8, 39.5, 43.5, 54.1, 58.5, 61.8, 131.7, 132.0, 132.2, 132.3, 133.2, 133.3, 145.0, 145.1, 177.3, 178.0, 178.3, 178.4, 178.7, 178.9, 179.0, 179.3, 179.7, 179.8.

<sup>13</sup>C nmr BHAlyslys<sub>2</sub> derivative (D<sub>2</sub>O):δ 26.9, 27.1, 32.2, 32.3, 34.7, 34.8, 35.1, 35.3, 35.6, 35.7, 39.5, 43.4, 54.1, 58.6, 61.8, 131.7, 131.9, 132.2, 132.3, 133.3, 144.9, 145.0, 177.7, 178.4, 178.8, 179.0, 179.3, 180.0.

(SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> BRI2792

Trifluoroacetic acid (4ml) was added to a suspension of (SEQ ID NO: 2) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>DBL<sub>6</sub> (0.73g: 0.1mmol) in dry dichloromethane (4ml) under nitrogen. A vigorous evolution of gas was observed for a short time and the resulting solution was stirred at room temperature for two hours and then concentrated. The residual syrup was dissolved in water (5ml), the solution passed through a column of Amberlite IRA-401(OH) and the filtrate

concentrated to give (SEQ ID NO: 3) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> as a viscous oil (0.49g). The oil was redissolved in water (5ml) and N,N-dimethyl-N-allylamine buffer (pH 9.5; 3ml) added. Solid sodium 4-sulfophenylisothiocyanate monohydrate (1.30g; 5.1mmol) was then added and the resulting solution heated under nitrogen at 53 °C for two hours and then cooled. The solution was concentrated and the brownish solid residue purified by gel filtration (Sephadex LH20; water). The pure fractions were combined, passed through a column of Amberlite IR 120(Na) and freeze dried to give the sodium 4-sulfophenylthiourea terminated (SEQ ID NO: 3) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> dendrimer as a fluffy white solid (374mg). <sup>1</sup>H nmr (D<sub>2</sub>O): δ 1.40; 1.72; 3.08; 3.42; 4.24; 4.60; 7.30; 7.40 (d, J=9Hz); 7.78 (d, J=9Hz). <sup>13</sup>C nmr . (D<sub>2</sub>O): δ 27.3; 32.5; 35.9; 43.7; 48.9; 58.6; 63.3; 128.8; 131.0; 143.7; 144.7; 145.1; 177.7; 178.1; 183.8; 185.2.

Please replace the paragraph beginning on page 24 at line 8, with the following rewritten paragraph:

The corresponding (SEQ ID NO: 2) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub>, (SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> (**BRI2992**), and (SEQ ID NO: 5) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub>lys<sub>64</sub> (**BRI2993**) dendrimers terminated with 16, 64, and 128 sodium 4-sulfophenylthiourea groups respectively were similarly prepared.

Please replace the paragraph beginning on page 25 at line 13, with the following rewritten paragraph:

(SEQ ID NO: 3) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> **BRI2999**

Trifluoroacetic acid (2ml) was added to a suspension of (SEQ ID NO: 2) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>DBL<sub>6</sub> (0.73g; 0.1mmol) in dry dichloromethane (2ml) under nitrogen. A vigorous evolution of gas was observed for a short time and the resulting solution was stirred at room temperature for two hours and then concentrated. The residual syrup was dissolved in water (5ml), the solution passed through a column of Amberlite IRA-401(OH) and the filtrate concentrated to give (SEQ ID NO: 3) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> as a viscous oil (0.49g). The oil was redissolved in water (5ml) and N,N-dimethyl-N-allylamine buffer (pH 9.5; 3ml) added. Solid sodium 3,6-sulfophenylisothiocyanate (234mg; 0.60mmol) was then added and the resulting solution heated under nitrogen at 53 °C for two hours and then cooled. The solution was concentrated and the brownish solid residue purified by gel filtration (Sephadex LH20; water).

The pure fractions were combined, passed through a column of Amberlite IR 120(Na) and freeze dried to give (SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> terminated with 32 sodium 3,6-disulfonaphthylthiourea groups as a fluffly off-white solid (119mg). <sup>1</sup>H nmr (D<sub>2</sub>O):δ 1.0-2.0; 3.18; 3.43; 4.31; 7.22; 7.80; 7.89; 8.25. <sup>13</sup>C nmr (D<sub>2</sub>O):δ 27.2; 32.4; 35.3; 43.7; 49.0; 58.5; 63.6; 128.4; 129.1; 131.4; 136.1; 136.6; 138.6; 139.0; 145.6; 178.4; 184.8; 186.7.

Please replace the paragraph beginning on page 27 at line 18, with the following rewritten paragraph:

The corresponding sodium 3,6,8-trisulfonaphthylthiourea terminated dendrimer (SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> **BRI 7011** was prepared similarly. The sweet potato sporamin vacuole

Please replace the paragraph beginning on page 30 at line 9, with the following rewritten paragraph:

(SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> **BRI 2922**

Trifluoroacetic acid (4ml) was added to a suspension of (SEQ ID NO: 2) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>DBL<sub>6</sub> (220mg; 30μmol) in dry dichloromethane (2ml) and the resulting solution stirred at room temperature under nitrogen for two hours and then concentrated. The residue was dissolved in dry DMSO (5ml) and the pH adjusted to 8.5 with triethylamine. Solid 4-nitrophenyl N,N,N-trimethylglycinate chloride (0.50g; 1.8mmol) was then added and the mixture stirred overnight at room temperature. The cloudy solution was then concentrated (50 /10<sup>-5</sup> mmHg) and the residue partitioned between water and dichloromethane. The aqueous layer was separated, washed with dichloromethane (3X) and ethyl acetate, and then concentrated to give an oil (1.128g). The crude product was purified by gel filtration (Sephadex LH20; water) to give the N,N,N-trimethylglycinamide terminated (SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> dendrimer (116mg). <sup>13</sup>C nmr (D<sub>2</sub>O):δ 25.5, 30.5, 30.8, 33.4, 42.1, 56.5, 57.1, 67.5, 68.1, 166.7, 167.0, 167.1, 176.0, 176.2.

Please replace the paragraph beginning on page 39 at line 31 with the following rewritten paragraph:

(SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>6</sub> [8-ocatanamido)- 5-acetamido-3,5-dideoxy-2-thio D-glycero- $\alpha$ -D-galacto-2-nonulopyranosidoic acid]<sub>32</sub> **BRI 6169**

Please replace the paragraph beginning on page 40 at line 3 with the following rewritten paragraph:

A solution of (SEQ ID NO: 3) BHA lyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub> (t-Boc)<sub>32</sub> (20.3mg.) in a mixture of trifluoroacetic acid (2ml.) and dichloromethane (2ml.) was stirred at 20 C for 2 hours then solvent was removed under vacuum. The residue was dissolved in dry dimethyl sulphoxide (1ml.) and di-isopropylethylamine (25mg.) and methyl [(8-octanoic acid N-hydroxysuccinimide ester) 5-acetamido-4,7,8,9-tetra-O-acetyl- $\alpha$ ,5-dideoxy-2-thioD-glycero- $\alpha$ -D-galacto-2-nonulopyranosid]onate (78mg.) were added. The mixture was stirred under argon at 20 C for 60 hours then solvent was removed under vacuum. The residue was dissolved in a freshly prepared 0.1M solution of sodium methoxide in methanol (2.5ml.) and the mixture stirred for 3 hours under argon at 20 C. The solvent was evaporated and the residue dissolved in water (1ml.) and stirred for 17 hours. This solution was subjected to size exclusion chromatography on Sephadex LH20 eluting with water. After lyophilisation, the product, (SEQ ID NO: 3) BHA lyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub> [(8-octanamido)-5- acetamido-3,5-dideoxy-2-thio-D-glycero- $\alpha$ - D-galacto-2-nonulopyranosidoic acid]<sub>32</sub> was obtained as a white powder 44mg. 86%.

Please replace the paragraphs on page 44 beginning at lines 5 and 23, respectively, with the following rewritten paragraphs:

(SEQ ID NO: 4) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub>

Trifluoroacetic acid (2ml) was added to a stirred suspension of (SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>DBL<sub>32</sub> (147mg) in dry dichloromethane (2ml) and the resulting solution stirred at room temperature under nitrogen for two hours and then concentrated. The residue was dissolved in N,N-dimethyl-N-allylamine buffer (pH 9.5; 5ml) and then solid 3,6-disulfonaphthyl isothiocyanate (400mg) added. The pH of the mixture was then adjusted to 9.5 by the addition of 1M sodium carbonate and the solution heated at 53°C for three hours under nitrogen. The reaction mixture was concentrated and the residue redissolved in water and the

solution passed through a column of Amberlite IR 120 (Na). The filtrate was concentrate was concentrated to give the crude product, which was purified by gel filtration (Sephadex LH20; water) to give (SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> with 64 sodium 3,6-disulfonaphthylurea groups as a white fluffy solid (175mg).

(SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub>

Trifluoroacetic acid (3ml) was added to a stirred suspension of (SEQ ID NO: 3) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>DBL<sub>32</sub> (300mg; 0.02mmol) in dry dichloromethane (3ml) and the resulting solution stirred at room temperature under nitrogen for two hours and then concentrated. The residue was dissolved in water and the solution passed through a column of Amberlite IRA 401 (OH) and the filtrate concentrated to give a viscous oil (187mg). The oil was dissolved in a 1:1 mixture of pyridine/water (8ml) and solid sodium 3,5-disulfophenyl isothiocyanate (680mg; 2mmol) added. The resulting solution was heated at 53°C for three hours under nitrogen. The solution was then concentrated to give a white solid residue. The crude product was purified by gel filtration (Sephadex LH20; water) to give (SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> with 64 sodium 3,6-disulfophenylurea groups as a white fluffy solid.

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Please replace the paragraph on page 45 beginning at line 11 with the following rewritten paragraph:

(SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> BRI 6741

Trifluoroacetic acid (3ml) was added to a stirred suspension of (SEQ ID NO: 3) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>DBL<sub>32</sub> (300mg; 0.02mmol) in dry dichloromethane (3ml) and the resulting solution stirred at room temperature under nitrogen for two hours and then concentrated. The residue was dissolved in water and the solution passed through a column of Amberlite IRA 401 (OH) and the filtrate concentrated to give a viscous oil (186mg). The oil was dissolved in a 1:1 mixture of pyridine/water (8ml) and sodium 3,5-dicarboxyphenyl isothiocyanate (450mg; 2mmol) added. The resulting solution was heated at 53°C for 13 hours under nitrogen. The solution was then concentrated to give a white solid residue. The crude product was purified by gel filtration (Sephadex LH20; water) to give (SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> with 64 sodium 3,6-dicarboxyphenylurea groups as a white fluffy solid.

Please replace the paragraphs on page 46 beginning at lines 5 and 23, respectively, with the following rewritten paragraphs:

(SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> BRI 6181

Trifluoroacetic acid (2ml) was added to a stirred suspension of (SEQ ID NO: 3) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>DBL<sub>32</sub> (147mg; 0.01 mmol) in dry dichloromethane (2ml) and the resulting solution stirred at room temperature under nitrogen for two hours and then concentrated to give a viscous oil. The oil was dissolved in N,N-dimethyl-N-allylamine buffer (pH 9.5; 5ml) and solid 4-phosphonooxyphenyl isothiocyanate (250mg) added. The pH of the resulting solution was adjusted to 10 with 1M sodium carbonate and the mixture heated at 53°C for three hours under nitrogen. The solution was then concentrated to give a white solid residue. The residue was redissolved in water and the solution passed through a column of Amberlite IR 120 (Na) and the filtrate concentrated. The residue was then purified by gel filtration (Sephadex LH20; water) to give (SEQ ID NO: 4) BHAllyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> with 64 sodium 4-phosphonooxyphenylurea groups as a white fluffy solid (150mg).



(SEQ ID NO: 4) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub>

Trifluoroacetic acid (2ml) was added to a stirred suspension of (SEQ ID NO: 3) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>DBL<sub>32</sub> (147mg; 0.01 mmol) in dry dichloromethane (2ml) and the resulting solution stirred at room temperature under nitrogen for two hours and then concentrated to give a viscous oil. The oil was dissolved in N,N-dimethyl-N-allylamine buffer (pH 9.5; 5ml) and solid 4-phosphonophenyl isothiocyanate (250mg) added. The pH of the resulting solution was adjusted to 9 with saturated sodium bicarbonate solution and the mixture heated at 53°C for three hours under nitrogen. The solution was then concentrated to give a white solid residue. The residue was redissolved in water and the solution passed through a column of Amberlite IR 120 (Na) and the filtrate concentrated. The residue was then purified by gel filtration (Sephadex LH20; water) to give (SEQ ID NO: 4) BHAlyslys<sub>2</sub>lys<sub>4</sub>lys<sub>8</sub>lys<sub>16</sub>lys<sub>32</sub> with 64 sodium 4-phosphonophenylurea groups **BRI 6196** as a white fluffy solid (152mg) after freeze drying.

Please replace the paragraph on page 50 beginning at line 18 with the following rewritten paragraph:

The saccharin-terminated (SEQ ID NO: 4) BHA.Lys.Lys<sub>2</sub>Lys<sub>4</sub>.Lys<sub>8</sub>.Lys<sub>16</sub>.Lys<sub>32</sub>... dendrimer **BRI-6189** was similarly prepared.